Acetaminophen Toxicity: Role of Metabolism

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Disclosures

- NIDDK- DK06799
- NICHD - HD31324
- STTR - R41 DK79387
- NIDDK - DK81403

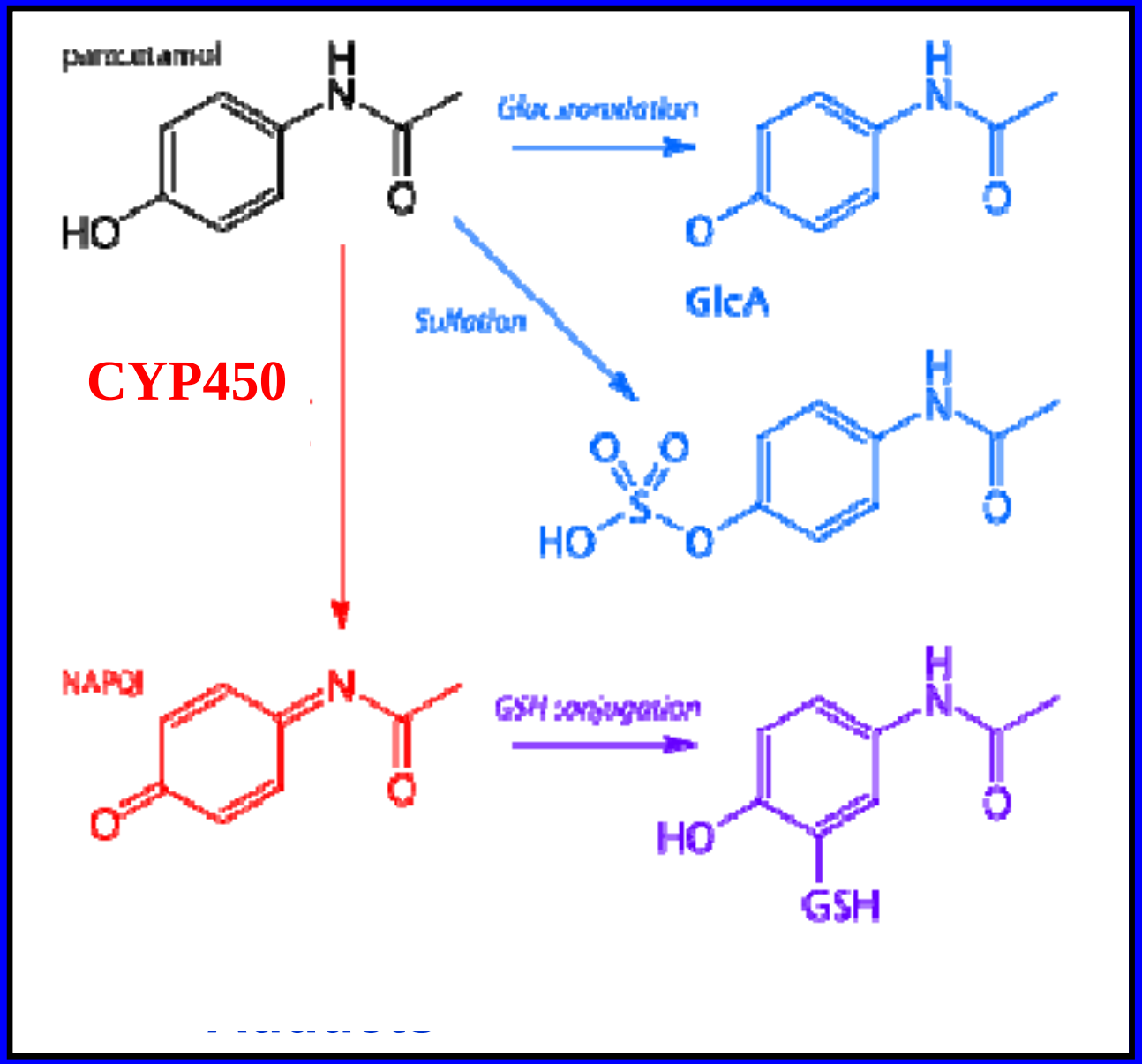
Role of Metabolism in Acetaminophen (APAP) Toxicity

- Liver injury from APAP initially reported in two patients in 1966
- Toxicity in murine model 1966, dose dependent hepatotoxin
- Mitchell, Jollow, Potter, Gillette, and Brodie (1970's)
- At high doses, APAP converted to a reactive metabolite that covalently bound to cysteine groups on proteins

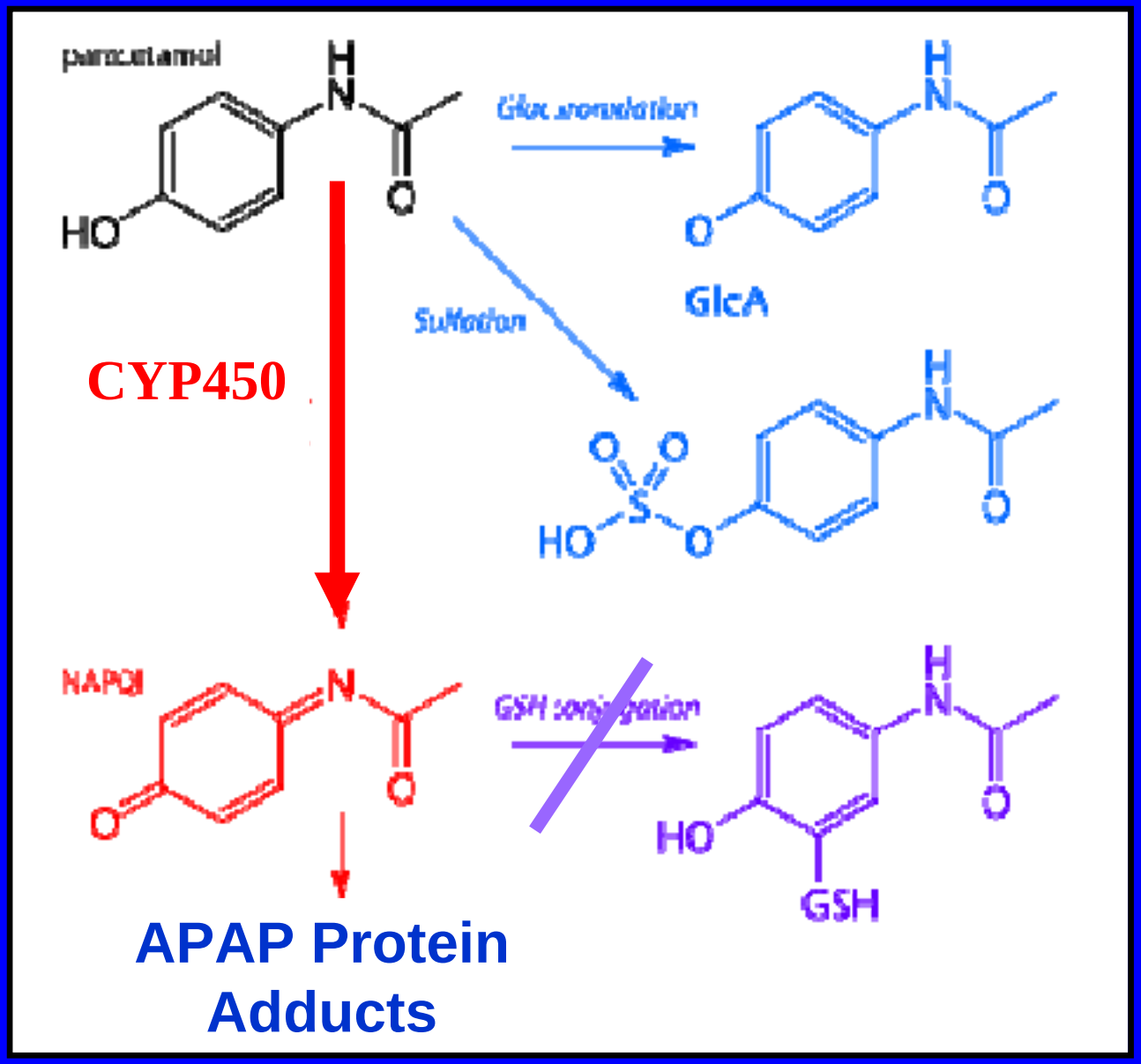
Metabolism and APAP Toxicity

- Degree of covalent binding correlated with toxicity
- Reactive metabolite identified to be NAPQI, formed by direct oxidation of APAP
- CYP2E1, CYP1A2, CYP3A4, and CYP2D6
- NAPQI reacts with glutathione through conjugation to form 3-glutathion-*S*-yl-acetaminophen
- Administration of cysteine prevented the toxicity; development of N-acetylcysteine (NAC)

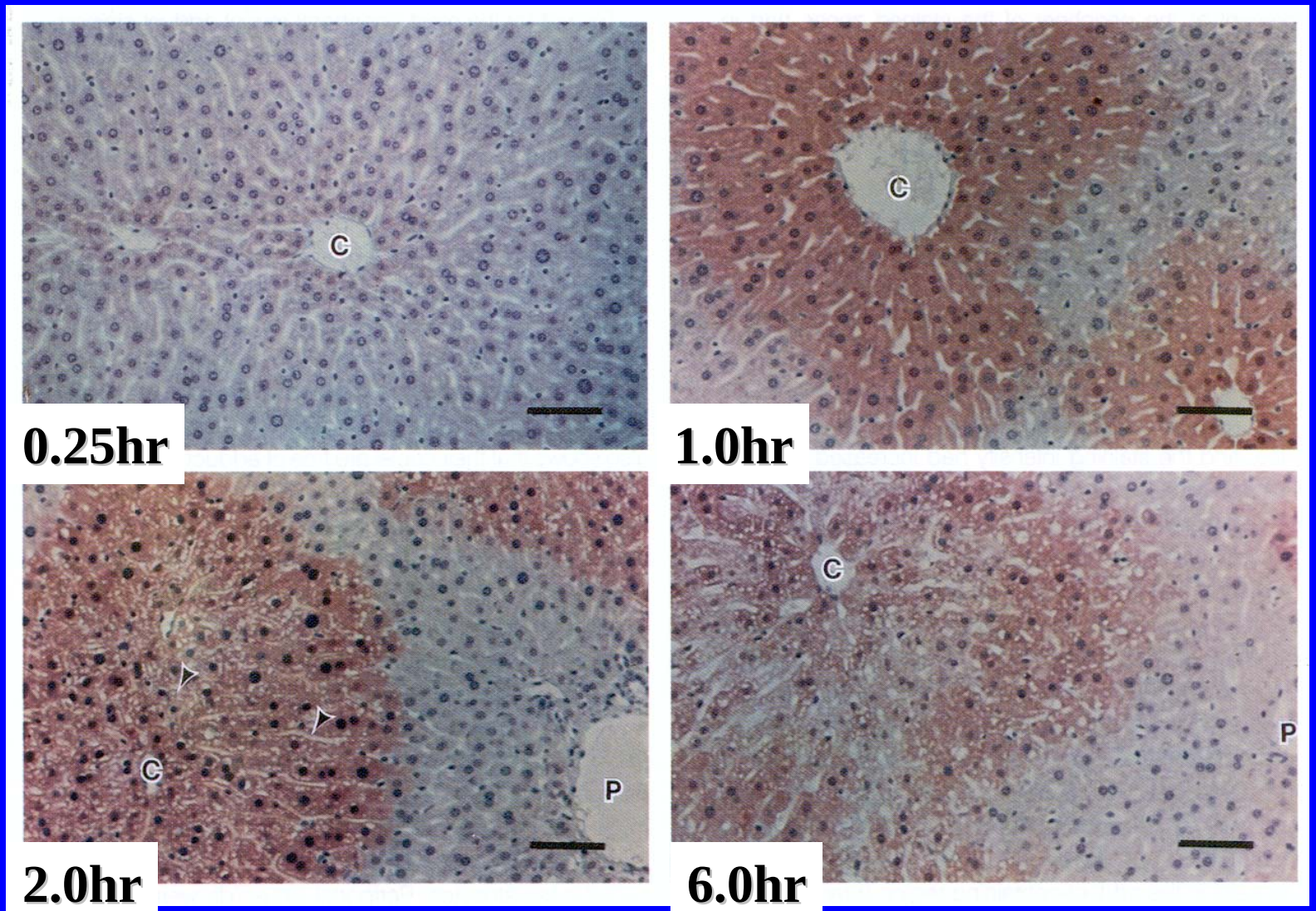
Role of Metabolism in APAP Toxicity



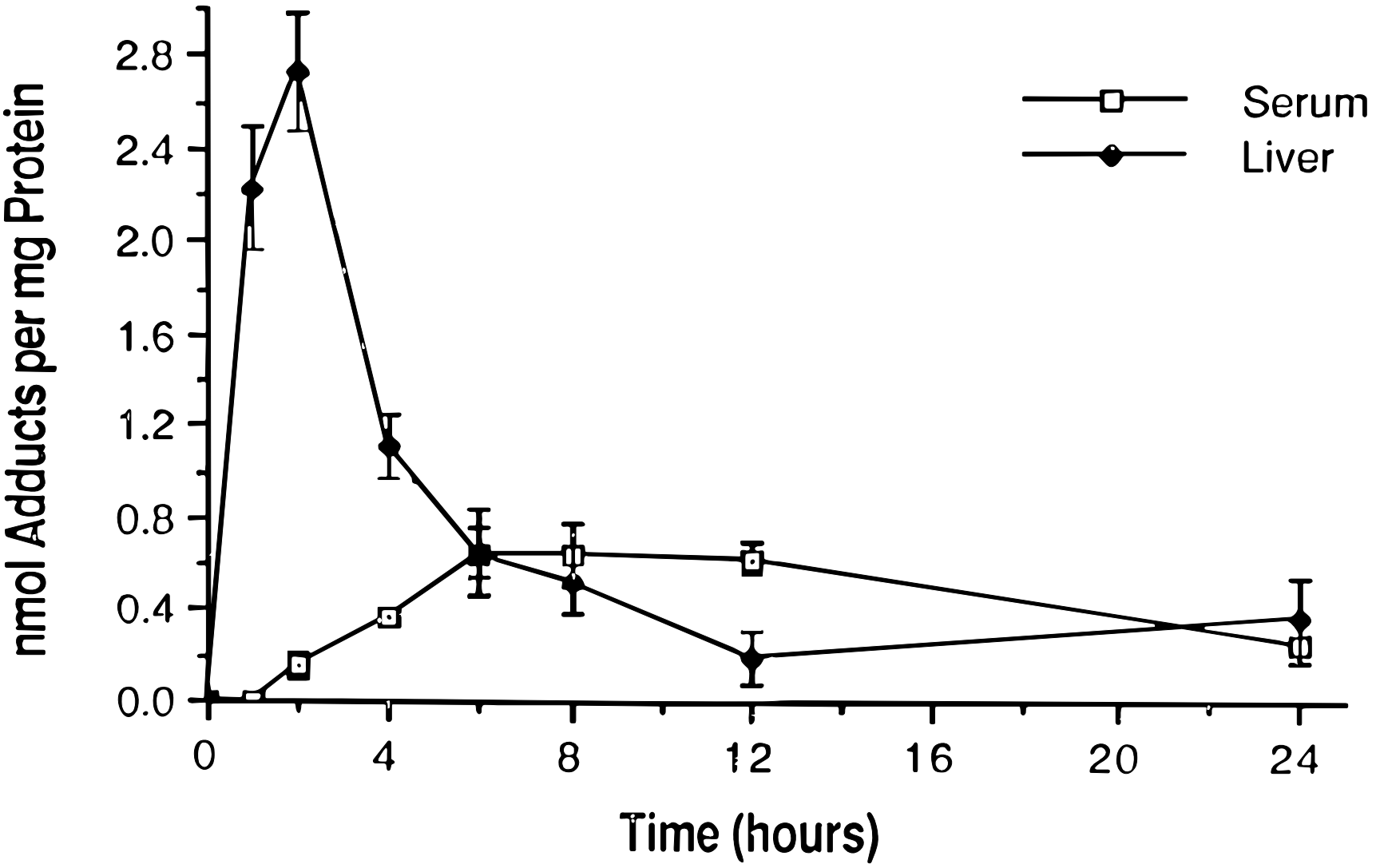
Role of Metabolism in APAP Toxicity



APAP Protein Adducts in Mice: Immunohistochemical Studies



Roberts et al, 1991, Am J Path


nmol Adducts per mg Protein
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0.0
0
4
8
12
16
20
24
Time (hours)
Serum
Liver

Adduct Data in Mice

- Dose dependency
- Appearance of adducts in liver preceded the elevation of hepatic transaminase values in serum
- Treatment of mice with NAC reduced toxicity and hepatic protein adducts

Other Mechanisms of APAP Toxicity

- Oxidative Stress
- Inflammatory Response
- Mitochondrial Permeability Transition
- Downstream events that occur after metabolism

?

What does this mean for patients?

Early Adduct Data in Humans – Assay Limitations

- Hinson et al (1990) - ELISA
  - 11 patients at risk for APAP toxicity
  - 5/11 received NAC within 8 hours, no toxicity, no adducts
  - 6/11 received NAC after 8 hr; significant toxicity; adducts detected
- James et al (2001) – Western Blot
  - 51 children with acute overdose
  - 6 patients with ALT > 1000 IU/L
  - Adducts in 1 patient (ALT > 8000 IU/L)

Lancet 1990, Hinson JA et al; J Clin Pharm 2001, James et al.

Acetaminophen Protein Adduct Assay

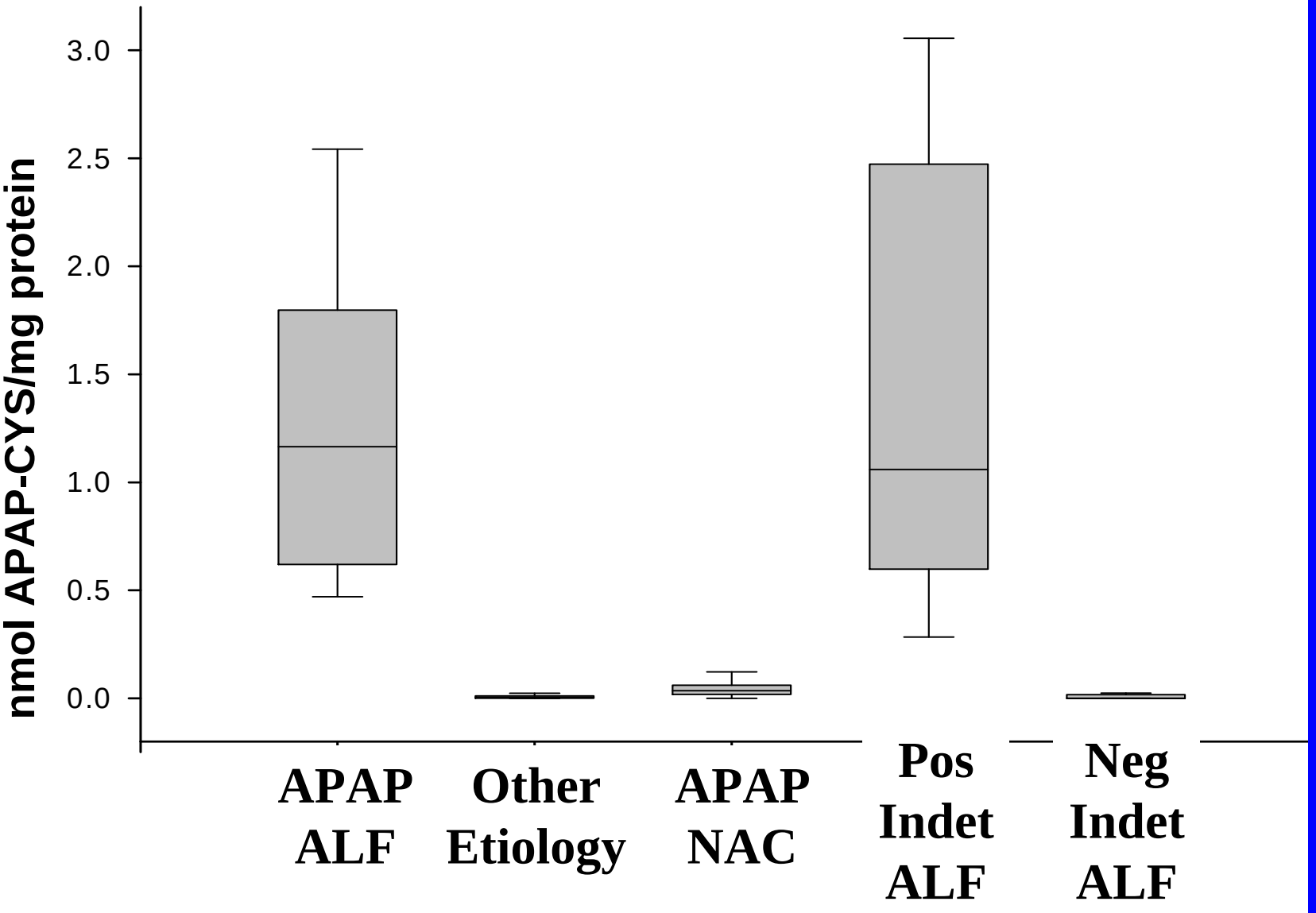
- Serum sample
- Removal of small molecules
  - Parent, metabolites
- Protease digestion
- HPLC-EC analysis to detect APAP cysteine adduct
- Quantified relative to a standard curve

Drug Metab Dispos 2002;30(4):446-451

Measurement of serum acetaminophen-protein adducts in patients with acute liver failure (ALF)

Davern TJ, James LP, Hinson JA, Polson J, Larson AM, Fontana RJ, Lalani E, Munoz S, Shakil AO, Lee WM. *Gastroenterology* 2006;130(3):687-94.

- **Three patient groups**
  1. Known APAP cases
     - Acute Liver Failure
     - Acute overdose with early treatment
  2. Known Cases of Other Etiology of ALF
  3. ALF Cases of Indeterminate Etiology of ALF
- **Analysis of samples in blinded fashion**



Gastroenterology 2006;130(3):687-94

ALF of "Indeterminate Etiology"

- Samples measured from 36 patients with ALF of indeterminate etiology
  - Extensive testing for viral hepatitis, autoimmune disease, etc
- 7 of 36 positive for APAP-CYS adducts **(19.4%)**
- Children with ALF of indeterminate etiology -- 12.5% of cases of indeterminate

Pediatrics* **118:e676-e681.

Pharmacokinetics of Acetaminophen-Protein Adducts in Adults with Acetaminophen Overdose and Acute Liver Failure

James LP, Letzig L, Simpson PM, Capparelli E, Roberts DW, Hinson JA, Davern TJ, Lee WM

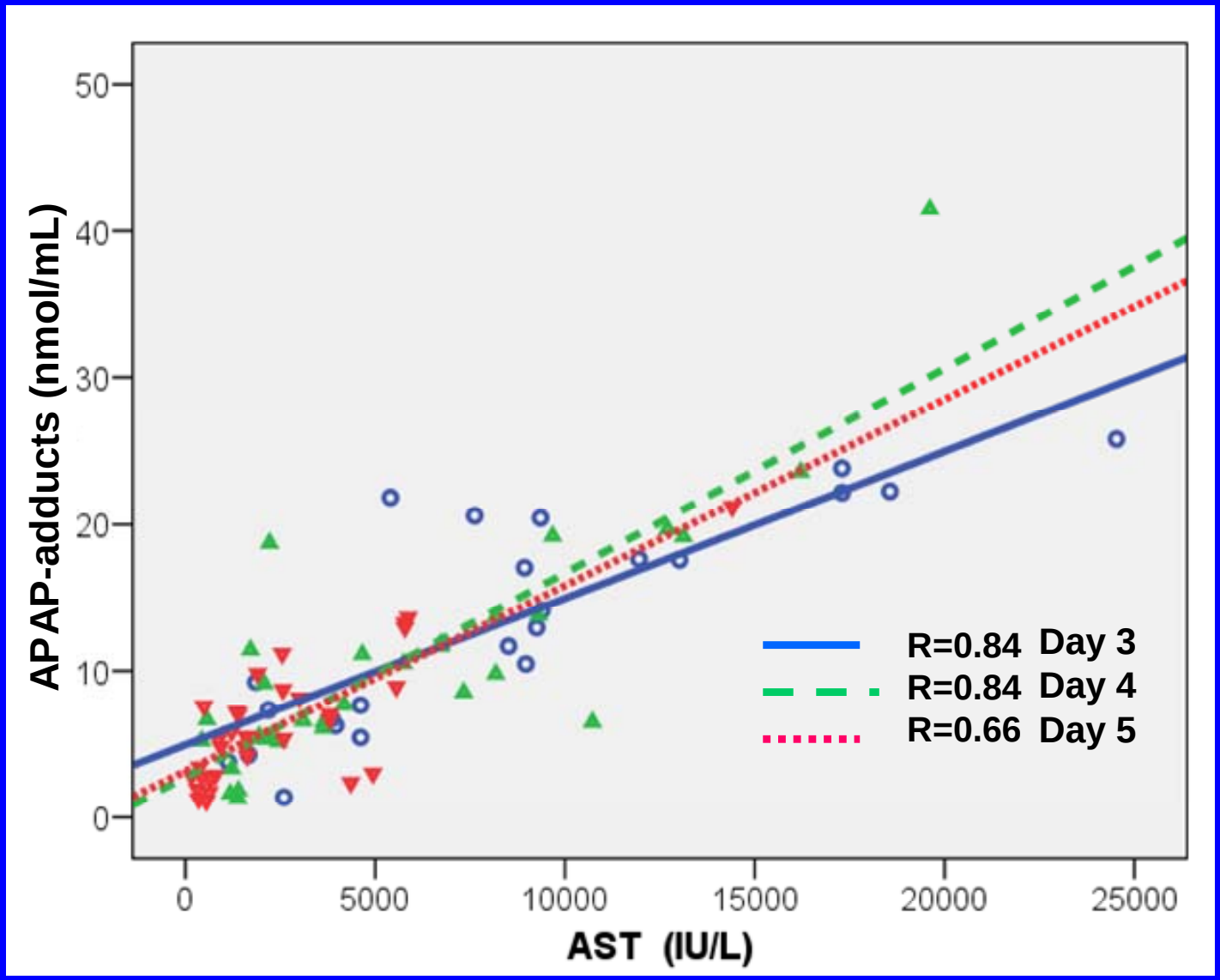
Drug Metab Dispos 2009;37(8):1-6

May 13, 2009; DOI: 10.1124/dmd.108.026195

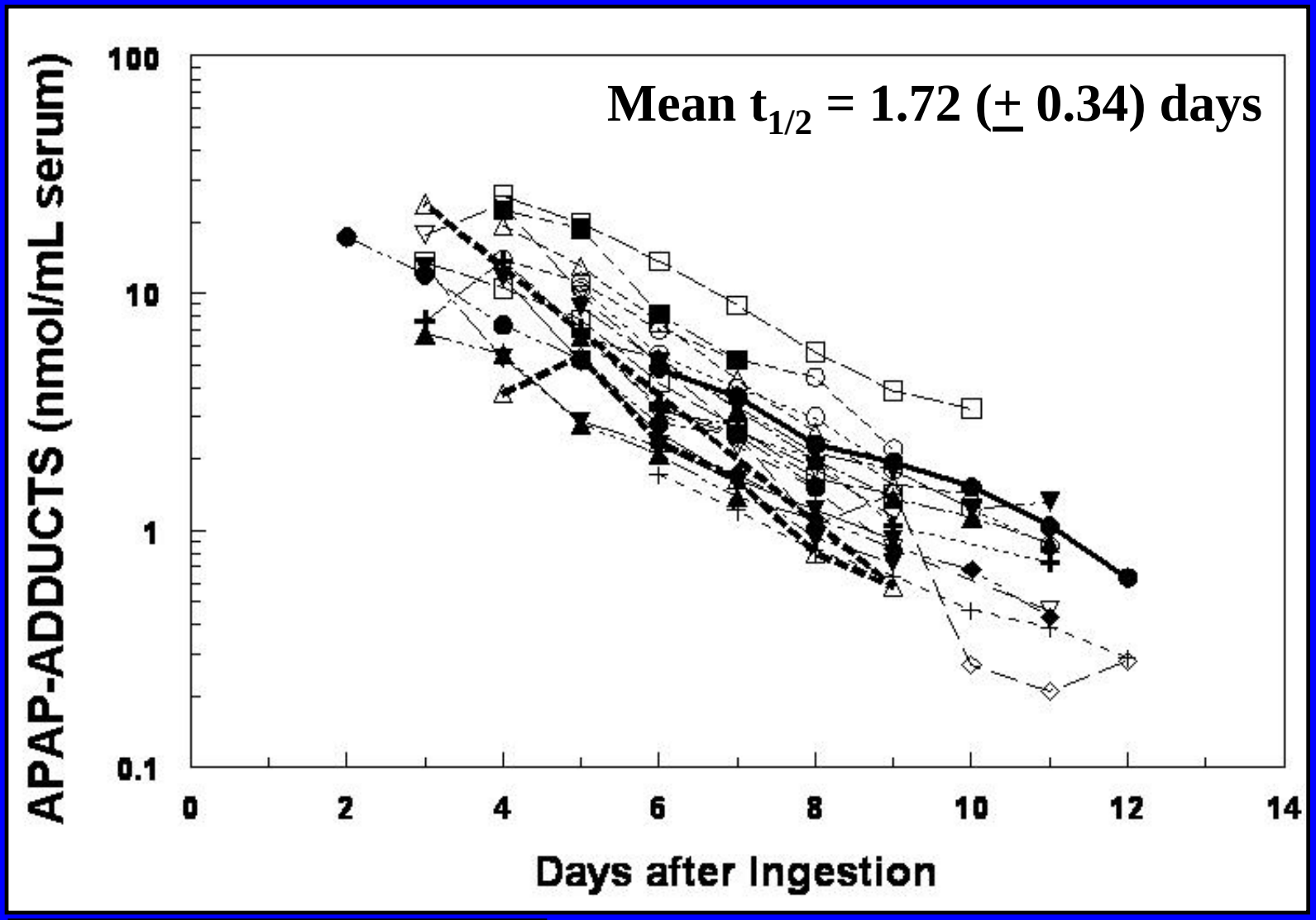
Adults with APAP Induced Acute Liver Failure

- 53 adults
  - (1) presence of coagulopathy (INR ≥1.5)
  - (2) evidence of hepatic encephalopathy
  - and (3) presentation within 26 weeks of illness onset without evidence of previous liver disease.
  - APAP Diagnosis
    - > 4 grams/day within 7 days of presentation
    - Detection of APAP on admission, or
    - ALT > 1000 IU/L, history of APAP use, regardless of level

Correlation of Adducts with AST in Adults with Acute Liver Failure



Detection of Adducts for 12 Days After APAP Overdose



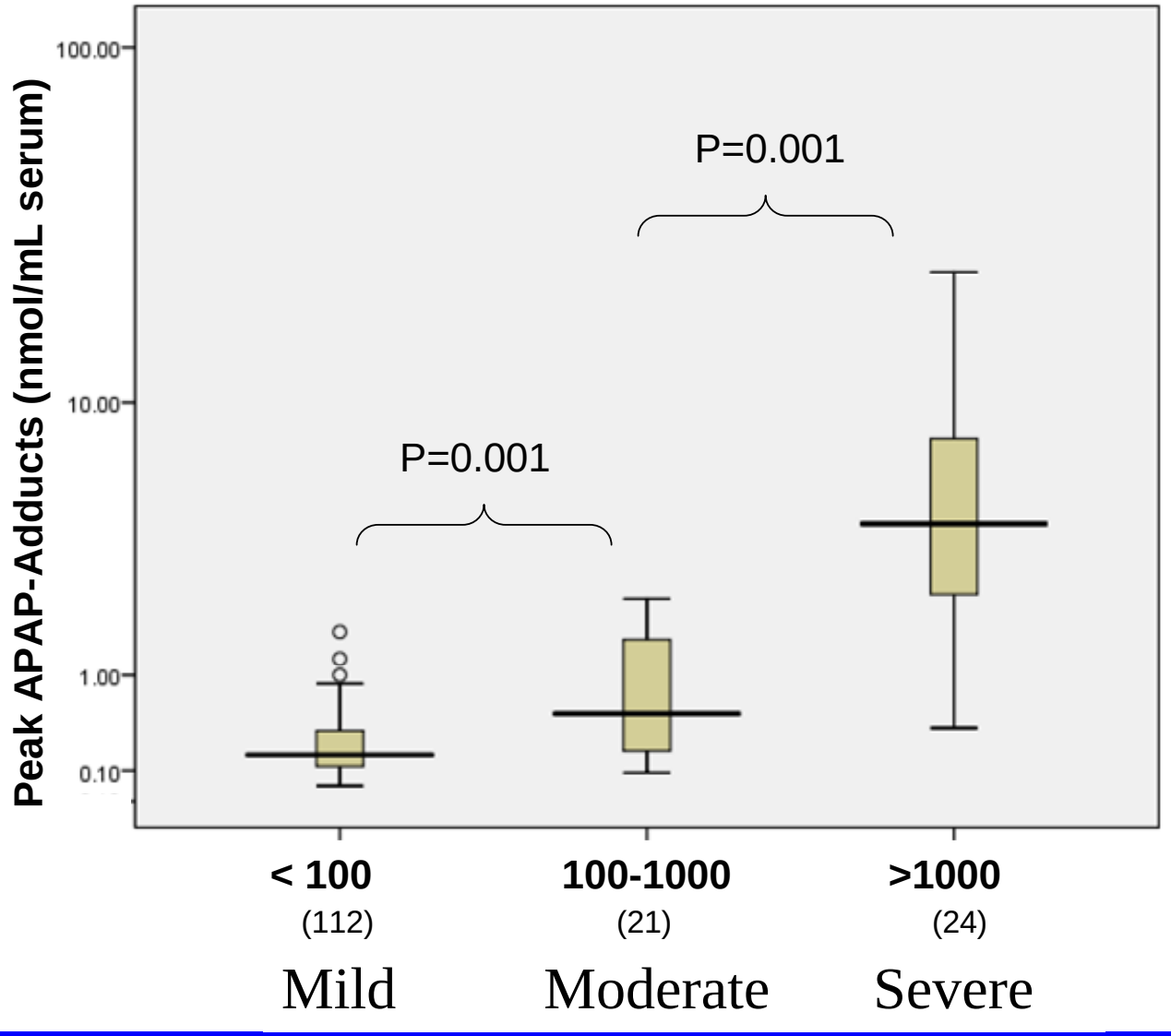
N=18; 4+ samples available

Pediatric Data

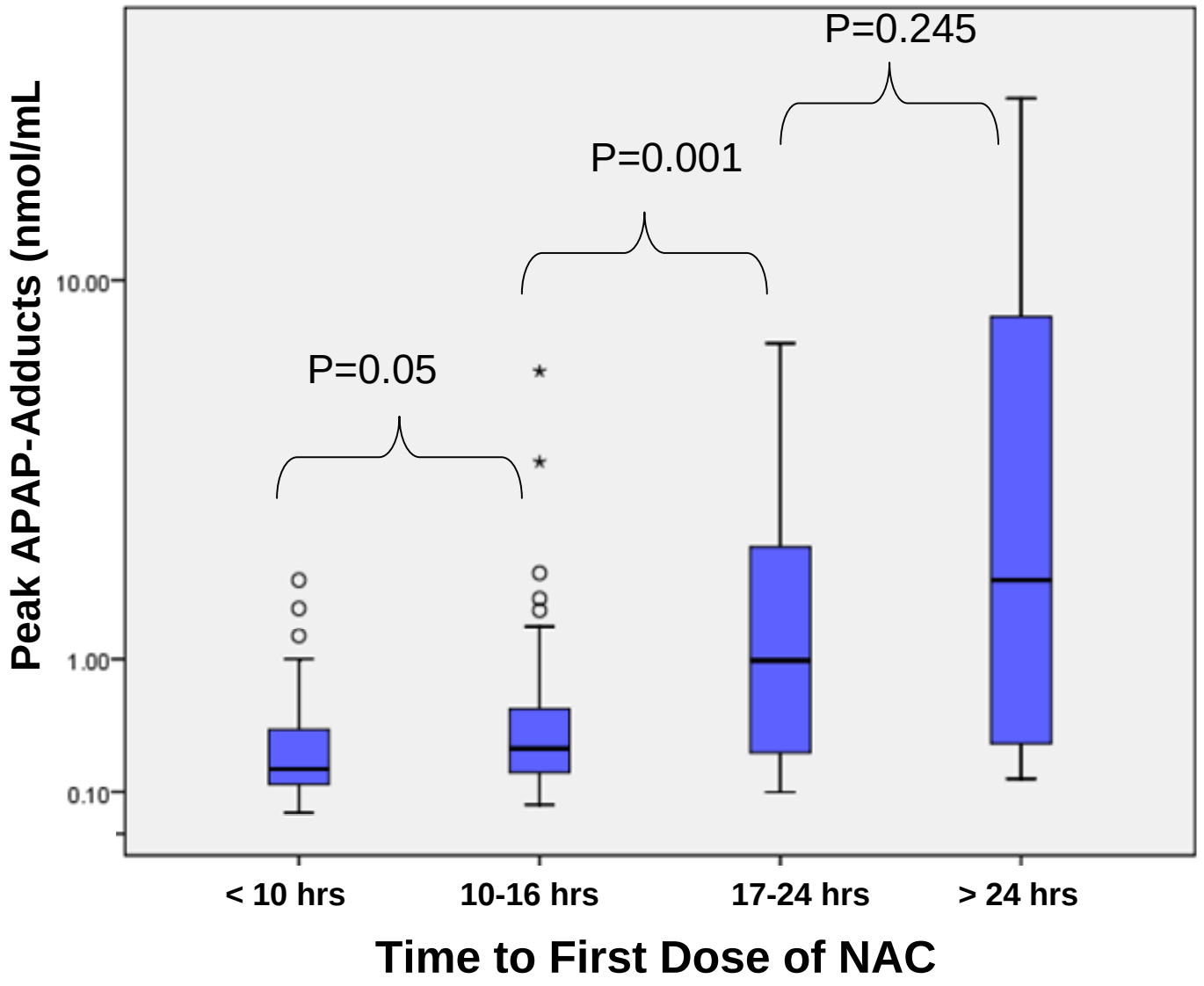
- 157 children/adolescents with acute overdose
- Convenience sampling approach
- Network of Pediatric Pharmacology Research Units, NICHD
- 80% female; 83% adolescents
- > 90% treated with N-acetylcysteine

Clin Pharm Ther 2008;84:684-690

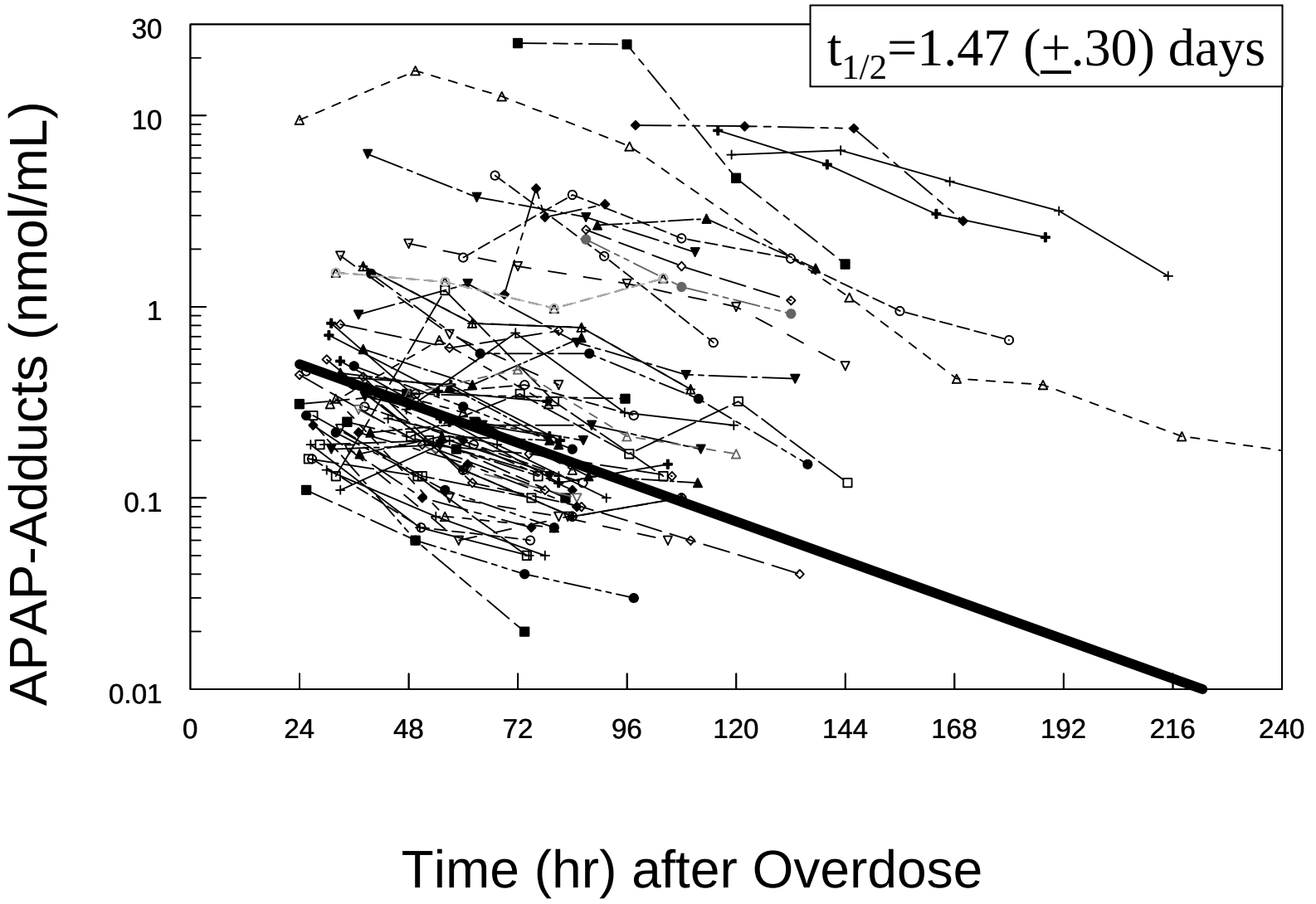
Adducts By Toxicity Severity



Adducts By NAC Treatment

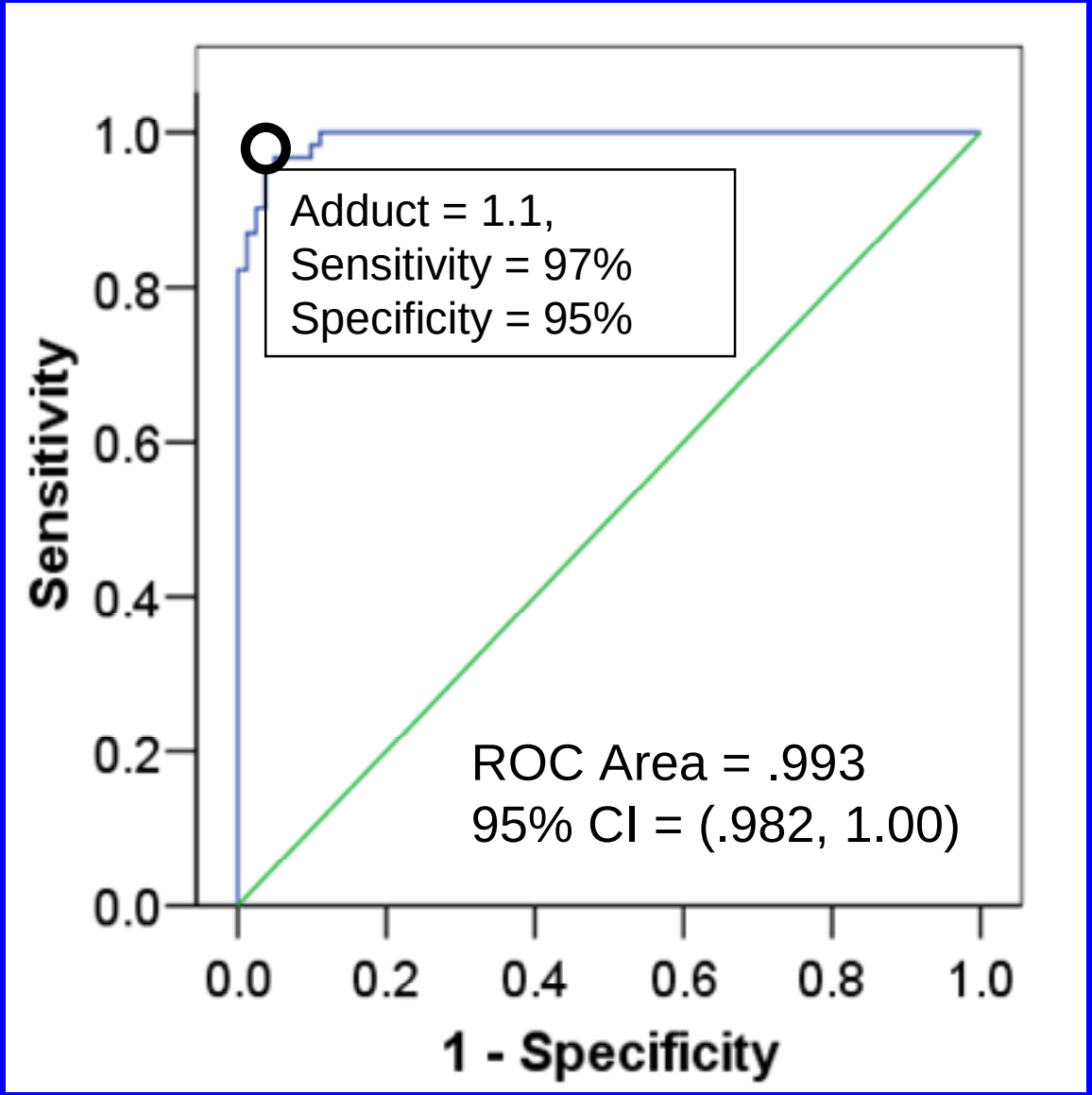


Adduct Elimination in Children



Raw data from subjects with ≥ 2 samples

Receiver Operator Curve Analysis of Adduct Levels To Establish Sensitivity and Specificity

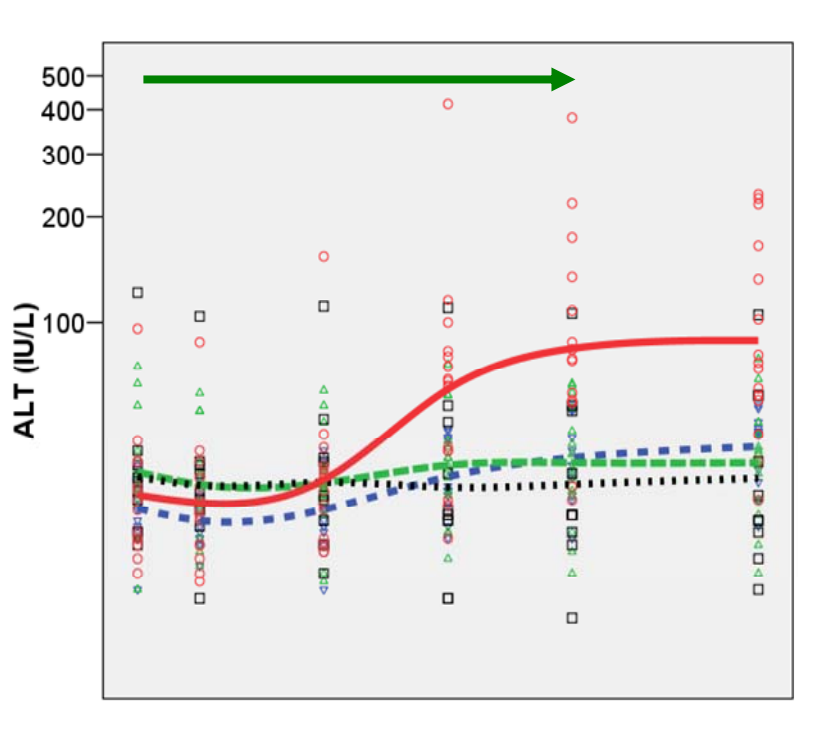


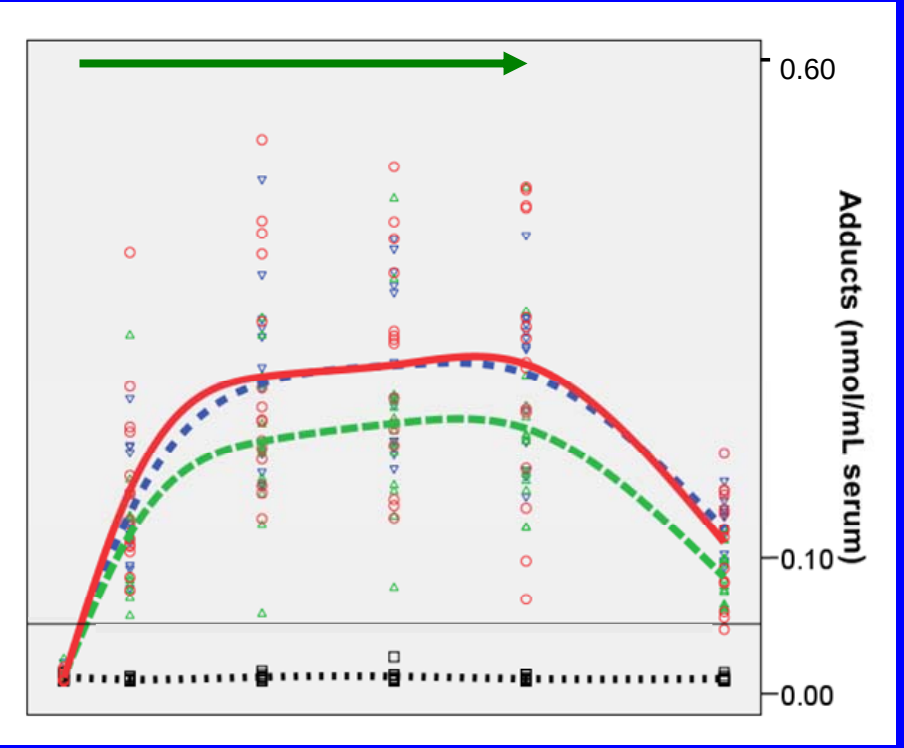
Healthy Adult Volunteers Receiving APAP

- 52 volunteers housed as inpatients for 14 days.
- Received APAP (4 grams daily) over 7 days; Placebo group
- Patients stratified by change in ALT from baseline:
  - **Responders**: > 2 fold elevation
  - **Non-responders**: < 1.5 fold elevation
  - **Intermediate responders** ≥ 1.5 and ≤ 2 fold elevation

Presented at the Annual Meeting of the American Association for the Study of Liver Diseases, 2007

ALT and Adduct Values in Adults Treated with APAP 4 grams/day

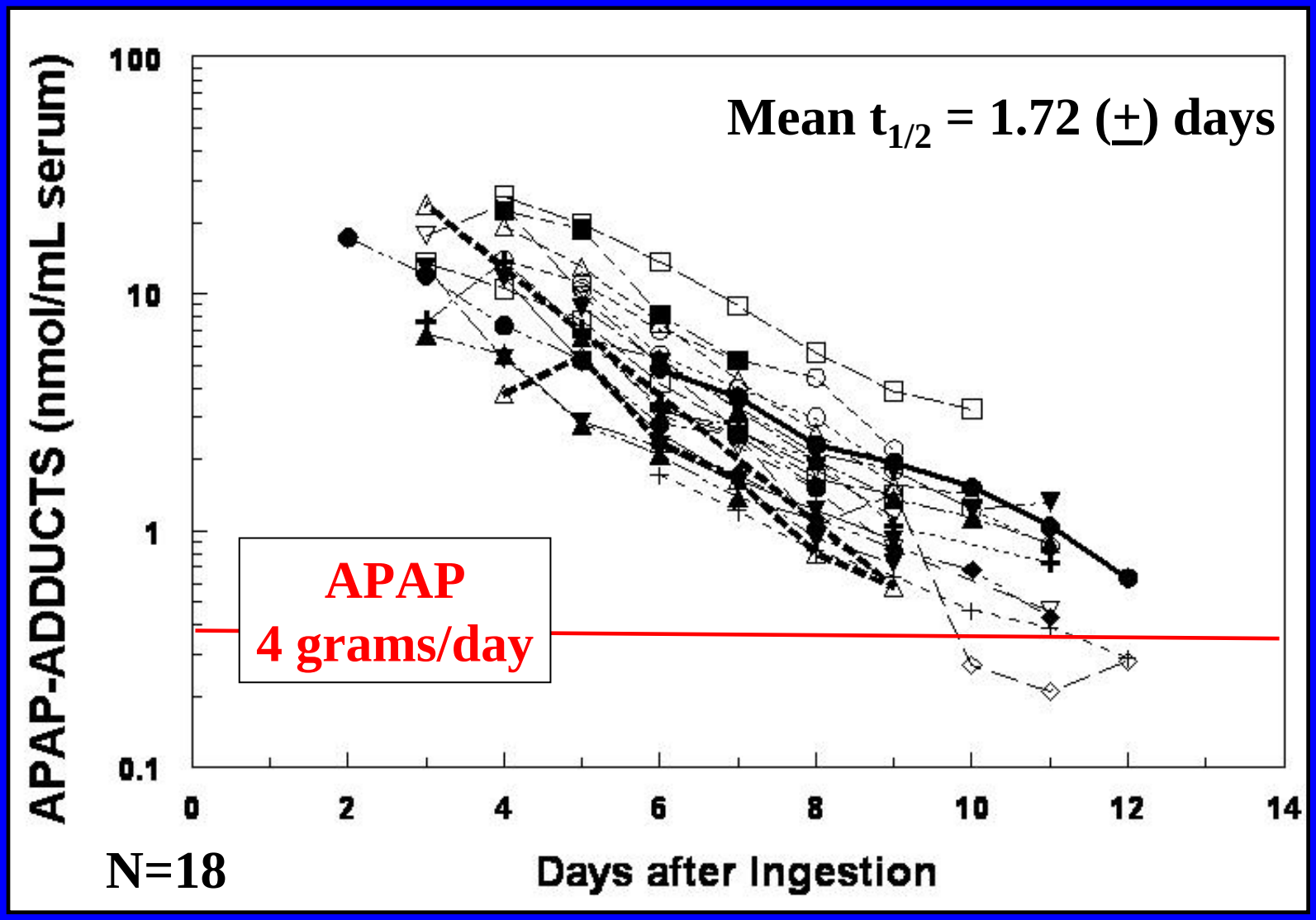




APAP 1 gram q 6 hrs

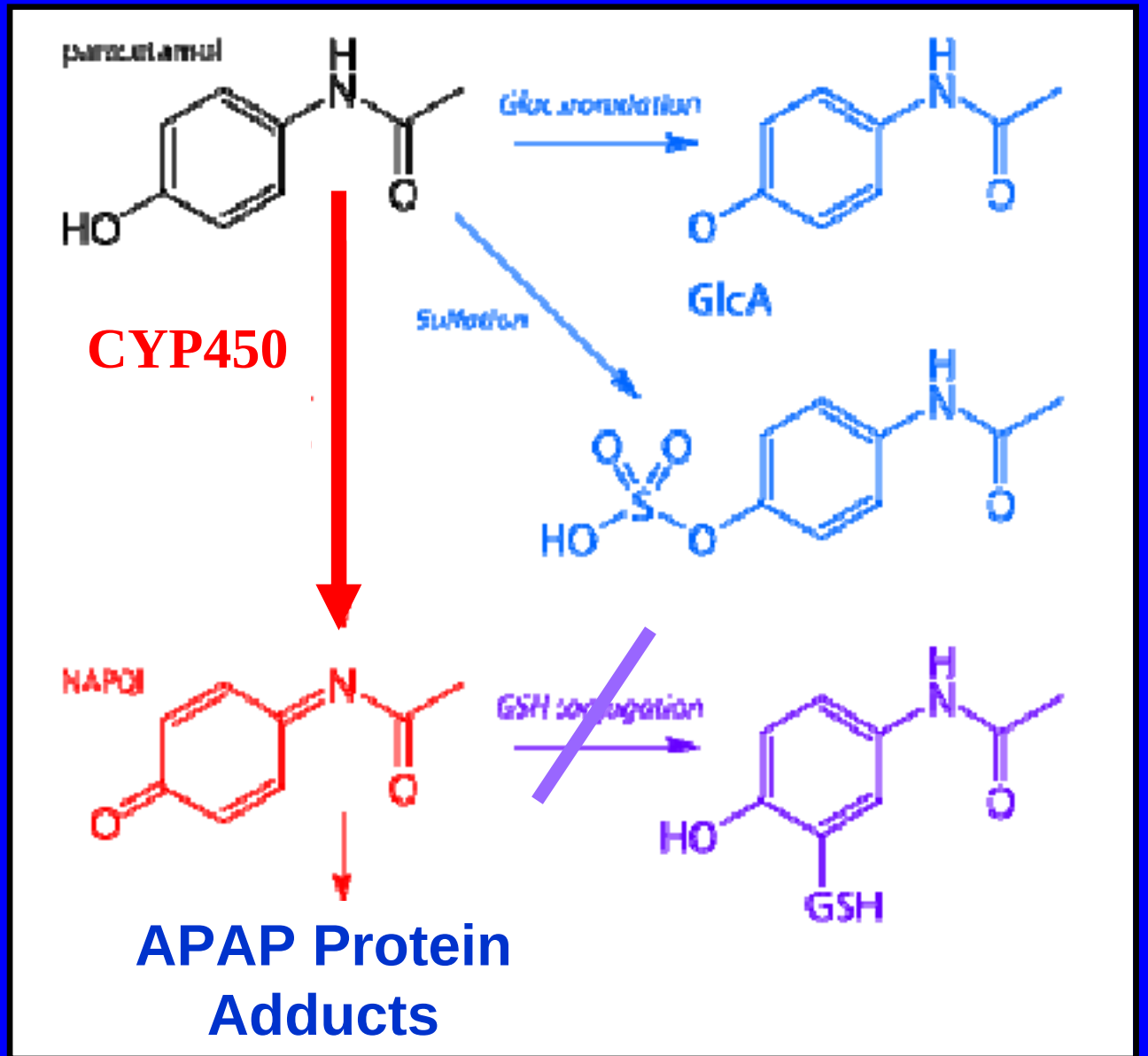
Placebo**, **Non-responder**, **Intermediate responder**, **Responder

Comparison of Adduct Levels Between Populations



Metabolism in APAP Toxicity

Patient Susceptibility



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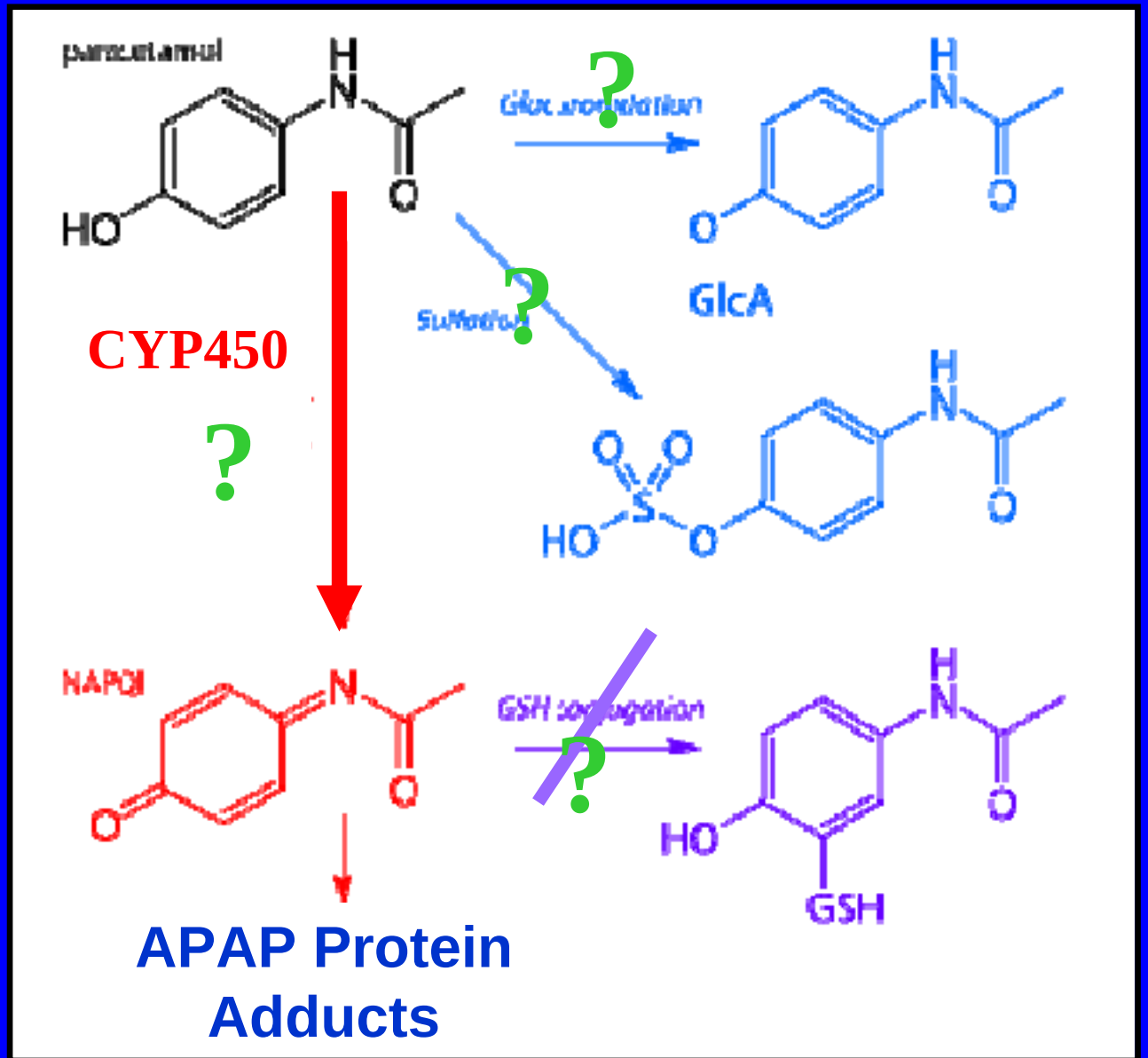
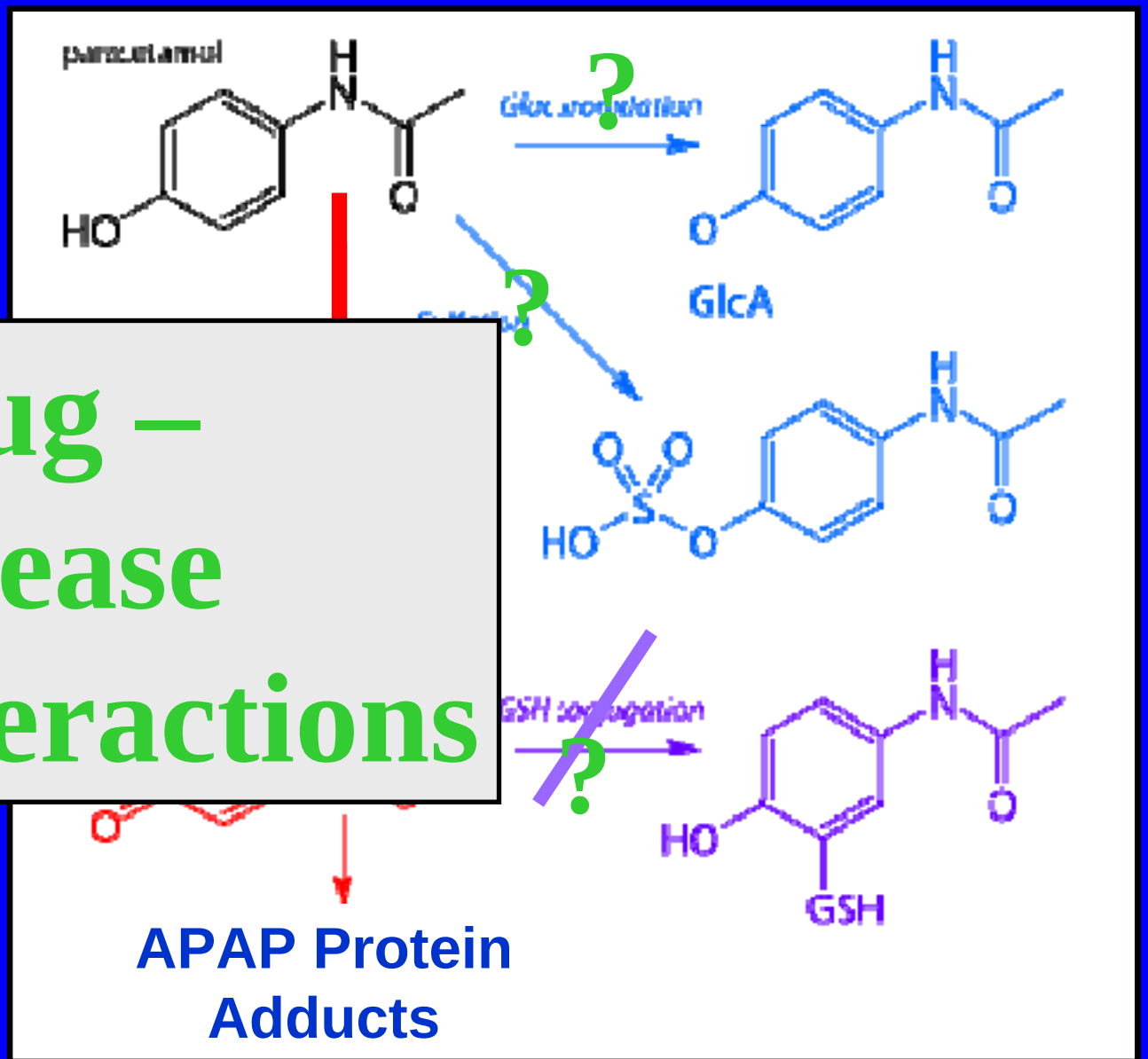


Metabolism in APAP Toxicity
Patient Susceptibility



Drug – Disease Interactions

Conclusions

- The toxicity of APAP is largely influenced by metabolism of the drug
- Acetaminophen protein adducts reflect metabolism of the drug and are a specific biomarker of APAP toxicity in patients
- The clinical significance of low levels of adducts in adults receiving 4 grams per day is unclear
- Proteomic studies in human samples are in progress
- Continued study of patient susceptibility needed (pharmacogenetics/disease/adaptive response)

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  - Kosair Children's Hospital, Louisville, KY
  - Rainbow Babies and Children's Hospital, Cleveland, OH
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